Subject Botany

M.Sc. (Semester II) Department of Botany

Course MBOTCC-6 Taxonomy Anatomy .&Embryology Unit -III

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What is Molecular systematics.

Molecular systematics is the use of **molecular** genetics to study the evolution of relationships among individuals and species. The goal of this branch of systematic studies is to provide insight into the history of groups of organisms and the evolutionary processes that create diversity among species using molecular data .

or

Molecular systematics can be defined as the use of the information contained in molecular data to reconstruct phylogenetic relationships. A phylogeny, or evolutionary tree, is the pattern of historical relationships among groups (lineages) of elements (e.g. organisms, sequences)

Molecular systematics allows the examination, of how the species have changed over evolutionary time, as well as of the relationships between species that have no common physical characteristics.

There are three major domains of life:

i)**Prokaryote**s (modern bacteria),

2)**Archae** bacteria (descendants of ancient bacteria), and

3)**Eukaryotes** (cellular organisms with nuclei and organelles).

All these organisms share a common ancestry of hundreds of millions of years. All species over time are connected to one another through a web of interlacing DNA as they reproduce, separate to become new species, and reproduce again. All organisms carry their ancestors' genetic information with them as a bundle in each cell, The more closely related organisms are to one another the more similar will be the contents of that bundle will stay over time. One of the important the job of a gene, is conservation. Conservation is the force that keeps a biological or genetic link between every species on earth

Both Molecular and morphological data is useful and necessary in systematics. These two types of data constitute independent and complementary sources of

information for cross-validating hypotheses about evolutionary patterns and processes at different levels of biological organization.

Importance of Molecular sytematics today .

Understanding this pattern of relationships is essential in comparative studies because there are statistical dependencies among elements sharing common ancestry. Phylogenetic analyses used to be restricted to studies of organism evolution, but today, they are a standard tool in broader fields of research, whether related to genomics, protein engineering, conservation biology, or pest control in agriculture. For example, phylogenies were used to study the timing and ancestry of the main pandemic strain of the human immunodeficiency virus (HIV) ,2009 swine-origin H1N1 influenza A , outbreak and more recently to investigate the origins and evolutionary genomics the Covid -19 pandemic.

TECHNIQUES USED

Of the various techniques that can be used for molecular systematics the analysis of sequence variation in:

1) DNA and 2) /or protein

has become the standard, and has been used in the vast majority of recent phylogenetic studies. Using DNA and amino acid sequences in molecular systematics has several advantages over traditional morphological approaches.

Protein-LevelAnalysis:

Proteins were the earliest biomolecules used to study phylogenetics. Initially, protein differences could be studied only at the grossest levels. **A-Allozymes and Isozymes** it was found that populations of organisms could be distinguished based on possessing different alleles (genetic sites) that made proteins possessing the same function but with different chemical structures. These enzymes were called isozymes. Isozymes can be separated and compared for size by employing a technique called gel electrophoresis.

B-Immunological Techniques Antibodies are biomolecules that are able to recognize and bind very specifically to other molecules. Biologists employ antibodies that specifically recognize molecules at the surface of cells to test relationships between species.Antibodies that recognize cell-surface molecules on one species should recognize those same molecules in closely related species, but

not from distantly related species, allowing a researcher to gauge similarity between species

DNA-Level Analysis

A.DNA-DNA Hybridizations The earliest approach to the use of nucleic acids in systematic studies involved the hybridization of nuclear DNA. In this method, the first step is the isolation of double-stranded DNA molecules. . RNA and protein are removed from DNA. Double-stranded DNA is reduced to a certain length by mechanical shearing. Treatment of the DNA with heat or alkali breaks the hydrogen bonds between the two strands and thus produces singlestranded DNA. When single strands of DNA from the same or different species are mixed together under appropriate conditions, they will associate to form double-stranded DNA if there is sufficient complementarity among nucleotides in the two strands. The capability of two complementary DNA strands to pair with one another can be used to detect similar DNA sequences in two different species.variations in genome size, polyploidy, and repeated elements.

B-Restriction Enzyme Analysis

Restriction enzymes are endonucleases that cut double-stranded DNA. Type-II restriction endonucleases are generally used for phylogenetic purposes. The number of fragments produced by restriction endonucleases depends on the number of recognition sites present in the molecule. Some changes in nucleotid sequence affect recognition sites for restriction enzymes. The appearance of different numbers of recognition sites may contribute useful data for comparing different species For comparative purposes, cpDNA from different organisms can be cut by several restriction endonucleases. The DNA fragments are separated on the basis of size by electrophoresis on agarose or polyacrylamide gels.

It is also possible to construct a linkage map using RFLPs, because they are inherited as codominant markers .

D-DNA Sequencing

DNA sequencing is a recent approach for systematics as for all of biology, but it has become one of the most commonly utilized methods of the molecular approaches for phylogenetic relationships). There exist two main methods of DNA sequencing; the Maxam Gilbert or chemical method and the Sanger dideoxy or enzymatic method; the latter is in more widespread use.

E- PCR and PCR-Based Fingerprinting Techniques Polymerase chain reaction (PCR) is preferred to cloning because this technique amplifies DNA directly without cloning into a vector. PCR by thermal cycling is an in vitro method that can be used to amplify a specific DNA fragment from small amounts of DNA template. This technique also allows the sequencing of DNA from herbarium specimens, museums or herbaria.

PCR-based fingerprinting techniques have been successfully developed for the estimation of genetic diversity among closely related species and among populations/individuals with a great diversity of techniques, such as randomly amplified polymorphic DNA (RAPD), arbitrary primed-polymerase chain reaction (APPCR) .DNA amplification fingerprinting (DAF) , microsatellites, amplified fragment length polymorphisms (AFLP), and amplified fragment length polymorphism based mRNA fingerprinting (AMF)

DNA Materials Used in Phylogenetic Studies

Nuclear Genome Genome size varies among different organisms. This aspect of the nuclear genome can provide considerable systematic and phylogenetic information.

2. Nuclear Ribosomal DNA

Ribosomal RNAs are both structural and functional parts of ribosomes. Ribosomal RNAs are transcribed from ribosomal DNAs as tandem repeat gene families, but they are never translated. Each member of this gene family encodes three separate molecules in the following order along the chromosome: 18S, 5.8S and 28S rRNAs. 16S, 23S and 5S rRNAs for bacterial cells, 16S and 12S for mitochondria and 16S, 23S, 5S and 4.5S rRNAs for chloroplasts are characteristic units. rDNA genes are inherited biparentally. The tandemly repeated multigene family of the

18S, 5.8S and 28S ribosomal RNAs have proven to be a useful tool for molecular evolutionary studies in eukaryotes.

3. **Mitochondrial Genome Mitochondrial (mtDNA)** is a closed circular duplex molecule. The size of mtDNA differs among organisms. While the mtDNAs of animals consist of about 16,000 to 18,000 base pairs, the mtDNAs in plants are relatively large and vary over extreme limits. The size, structure and gene order of mitochondrial genomes vary widely in angiosperms; therefore, restriction analysis involving a whole genome is very difficult. mtDNA has highly conserved evolutionary sequences. Some genes that are vitally important for cell life are located on mtDNA. The alteration rate for mtDNA is 2% of the nucleotides in one million years. The evolutionary history of the plant mitochondrial genome is poorly known, and the animal mitochondrial genome is the best known of any group of organisms .Recent phylogeographic studies on mtDNA sequences have revealed the evolutionary history of several organisms .

4. Chloroplast Genome Chloroplast DNA (cpDNA) is a closed circular molecule, like mtDNA. cpDNA is also replicated semiconservatively and inherited maternally Its size varies among different species, generally ranging from 130 to 160 kb. In some exceptional plants, this may range from 120 to 217 kb .Whole land plants have a general structural organization for the chloroplast chromosome. There are two regions in the molecule that are identical but in opposite orientation to each other. These regions include approximately 15% of the whole chloroplast genome. Two unical regions known as small and large single-copy regions are also located between these inverted repeat regions. Like mitochondria, the number of chloroplasts increases according to the needs of plants (i.e. photosynthesis and energy). For phylogenetic purposes, the analysis of multicopy genomes such as cpDNA and mtDNA has several advantages over the analysis of nuclear genes and their products, as it is less prone to DNA alterations through mutation, recombination and introgression.

ADVANTAGES OF MOLECULAR SYTEMATICS

Using DNA and amino acid sequences in molecular systematics has several advantages over traditional morphological approaches . the universality of the character types and states (yielding a more objective selection and definition of homology, i.e. a similarity in character states due to their inheritance from a common ancestor); the high number of characters available for analyses (yielding data with a better statistical performance); the high degree of variation in the substitution rates among genes and gene regions (providing different levels of variability for specific questions); our increasingly comprehensive knowledge of the molecular basis underlying sequence evolution and function (allowing the construction of more sophisticated models of the evolutionary process); and the relatively easy collection of the data from different taxa (even from very small tissue samples and by researchers that do not necessarily have taxon-specific expertise). In the last few years, a great
amount of sequence data has been generated for higher taxa, and this has definitely boosted the possibilities for comparative and phylogenetic studies.

DISADVANTAGES OF MOLECULAR SYSTEMATICS

However, phylogenetic inference from molecular data is not free from methodological problems and pitfalls.
For example, molecular sequence
data has a relatively low character state space (four states in the case of DNA 20 in the case of amino acids), which may entail a high probability of homoplasy (similarity in character states for reasons other than common ancestry, such as convergence, parallelism, and reversal) due to the saturation of the substitution process (e.g. two sequences might have the same character state at a given position just by chance and not due to common ancestry) In practice, this problem is particularly important for some methods of phylogenetic inference, such as parsimony. Furthermore, gene phylogenies do not necessarily match those of the organisms due to several evolutionary processes such as horizontal gene transfer, gene duplication and loss, and deep coalescence .

Homologous genes that were separated by a speciation event (when a species diverges into two separate species) are termed orthologous, whereas homologous

genes that were separated by a gene duplication event and occupy two different positions in the same genome are termed paralogous. One might expect a total match between the gene tree and species tree if the genes used to reconstruct the phylogeny are orthologous, but in practice, different sets of orthologous genes may yield different phylogenies because of analytical limitations and differences in the phylogenetic signal:noise ratio due to the unequal action of natural selection or genetic drift .In some cases, molecular data is irretrievable, such as in ancient fossil taxa, although some data has been obtained for 'recently' extinct organisms an it cannot as yet be used on its own to describe new species.

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